# Sustained diuretic effect of plantain when ingested by sheep

CA O'Connell[a*], HG Judson[b] and GK Barrell[a]

[a]*Faculty of Agriculture and Life Sciences, P O Box 84, Lincoln University, Lincoln 7647, New Zealand,* [b]*Agricom, P O Box 3761, Christchurch 8140, New Zealand*

**Corresponding author:* Email: kyrapup@hotmail.com

## Abstract

Narrow-leaved plantain (*Plantago lanceolata* L.) was investigated for its potential to act as a diuretic in sheep. For seven days, two groups of sheep (n = 8) were provided with either ryegrass or plantain diets that had similar water content. Dry matter in feed, fresh faecal weight, and faecal water content was measured daily, as well as volume, specific gravity and osmolality of urine. Urine volumes on Day 1 of the trial were significantly different to those of Days 2-6 of the trial ($P < 0.05$) so the results were analysed separately. Plantain sheep had a higher volume of urine (by 1.7 L, $P < 0.05$) on Day 1 than Ryegrass sheep and continued to produce about 0.5 L more urine each day than Ryegrass sheep for the remainder of the study period. Because intakes of feed and feed water content for the two diets were essentially equal, this study provides the first direct evidence to show that plantain causes a diuresis when it is ingested by sheep, possibly by reducing reabsorption of water in the kidneys.

**Keywords:** diuretic; plantain; sheep nutrition; urine production

## Introduction

In temperate grazing livestock systems there has been some replacement of traditional grass/clover pastures with those containing various admixtures of herbs. One such herb is the narrow-leaved plantain (*Plantago lanceolata* L.), a non-leguminous perennial. Although the folklore literature abounds with information about medicinal and other activities present in various herbs, very little has been published in the scientific media to support such claims. Despite plantain reputedly having diuretic properties (Rumball et al. 1997; Tamura et al. 2002), there appear to be only two scientific studies that have investigated effects of dietary plantain in sheep. Wilman and Derrick (1994) recorded an increase in daily urine production of lambs fed plantain compared with others that were fed ryegrass and Deaker et al. (1994) found that plantain increased kidney weight of ram lambs, suggestive of an effect of the herb on kidney function. Recently, there has been an increase in concern about urinary nitrogen concentrations, especially in grazing dairy cows, as the high values occurring in dairy-cow-urine patches contribute strongly to nitrogen leaching into soils and subsoil water aquifers. The possibility of providing ruminant animals with pastures that may alleviate the nitrogen leaching potential via increases in urine volume, and hence, decreased concentration of nitrogenous compounds, is likely to be of considerable interest to pasture-based livestock operators.

The present study was designed to investigate the possibility that plantain has diuretic properties upon ingestion by sheep. This was determined by comparison of urine production by sheep fed plantain with that of others fed ryegrass, where the total water intake in both cases was restricted to the water content of the feed only and was matched for the two diets.

## Methods and materials

*Sheep*

Animals used in this study were six-month-old New Zealand Romney ewe lambs that were grazed on predominantly ryegrass/white clover pasture near Lincoln, Canterbury, New Zealand. They were transferred indoors where they were held for seven days in individual metabolism crates without access to drinking water. The sheep were allocated to two dietary treatment groups (Ryegrass, Plantain, 8 animals per group) that were balanced for live weight (average 38 kg) and urine production (daily volumes measured in an earlier study) and held in two separate rooms with four animals from each group per room. Either freshly harvested (see below) plantain (*Plantago lanceolata*, cultivar Ceres Tonic) or perennial ryegrass (*Lolium perenne*, cultivars Base and Expo) was provided to the sheep individually so that each animal received a daily feed water allocation of close to 4 litres. Each day's individual sheep feed allowance was computed from the dry matter content of the previous day's feed to maintain the constant daily water allocation. Throughout the trial, the sheep did not have access to drinking water. Any refused feed was collected the next day and weighed, then discarded. All procedures involving these animals were approved by the Lincoln University Animal Ethics Committee.

*Feed harvesting*

Plantain (two-year-old crop) and ryegrass (one-year-old crop) were grown as monocultures in separate paddocks. Each crop had a density of 2000 kg DM/ha and was harvested at about 0830 h every day using a motorised forage harvester. The feeds were transported to the rooms where the sheep were housed, weighed out into individual meal aliquots, and placed in each sheep's feed bin within half an hour of being harvested. Two samples of each day's harvest were collected in perforated bags, weighed, and placed in a 90˚C oven for 24 h or until dry. These were then removed and re-weighed to determine dry matter percentage, the mean of each pair being recorded.

*Urine and faeces*

Urine and faecal matter were collected separately into collection trays and buckets situated under the grating of each metabolism crate. Plastic mesh sheeting trapped the faeces to separate it from the urine. The urine was acidified by adding 250 mL of 5% sulphuric acid to the collection trays. Each morning, daily urine volume (i.e., previous day's) for each sheep was measured and a sample stored in a freezer (-20°C). Trays were rinsed with tap water, returned to the crates, and remained there until a fresh urine sample (circa 70 mL) urine was obtained. Acid was then placed in the tray for the remainder of the daily urine collection. The specific gravity of fresh urine was measured with a portable refractometer (Uricon, Atago USA Inc, Kirkland, WA, USA) within an hour of the animal urinating. Fresh and acidified urine samples were bagged separately in plastic bags and stored frozen (-20°C) for subsequent analyses. The frozen, fresh urine samples were later thawed for measurement of their osmolality (mmol/kg) with a vapour pressure osmometer (VAPRO 5520, Wescor Inc., Logan, Utah, USA) and multiplying this value by the daily urine volume gave the total osmols excreted per day in the urine.

The metabolism crates were cleaned daily to collect all faecal matter and each sheep's daily faecal production was weighed. A small sample was set aside to be freeze dried for subsequent analysis. The remainder was divided between two perforated plastic bags which were weighed and placed in 90°C ovens until dry. Once dry, the bags were re-weighed and the dry matter percentage calculated. This gave two estimates of faecal dry matter percentage (DM%) for each sheep's daily output. The mean of these two values was applied to the total faecal weight to determine daily faecal water volume. Daily fluid balance for each animal was measured as the difference between volume of water ingested in the feed (total water in) and the sum of urine volume plus faecal water volume (water out).

*Blood*

Blood samples (10 ml) were collected from all 16 sheep immediately prior to commencement of the study and again on completion using evacuated plastic tubes containing potassium ethylene diamine tetra acetic acid (K2E, B D Vacutainer®, Becton Dickinson and Company, Franklin Lakes, NJ, USA) as anticoagulant. Packed cell volume was determined in duplicate by the microhaematocrit procedure and the concentration of red blood cells in each blood sample was determined in duplicate using a haemocytometer. The mean of each pair of these measurements was recorded for each sheep. Sodium concentration of plasma was determined at a commercial analytical laboratory (Gribbles Veterinary, Christchurch, New Zealand) using an automated analyser equipped with a $Na^+$-sensitive electrode.

## Statistical analysis

Data were computed as a mean for each day. Results for Days 2-6 were calculated as the mean of all the daily data for each group (Plantain and Ryegrass) from the period, as determined using a pivot table in Microsoft Excel 2013. The data were analysed using Student's t-test to determine which input and output variables differed between the treatment groups. Each variable was assessed independently. This was completed using a one-way ANOVA from the computing software Minitab® 17. The data were further analysed to see if the difference in urine volume was still significant after accounting for difference in input values, dry matter in feed and water in feed, between the treatment groups. This analysis was carried out with an analysis of unbalanced design in GenStat, Version 16, using dry matter in feed as a covariate. The regression model is

$$Y_t = \mu + \alpha_t + \beta x + e$$

Where $\mu$ is the grand mean, $\alpha$ is the treatment effect of the diet, $x$ is a covariate for dry matter in feed, and $e$ is the error. The accumulated analysis of variance shows that the treatment effect remains significant ($P < 0.001$) after accounting for the variation due to differences in dry matter.

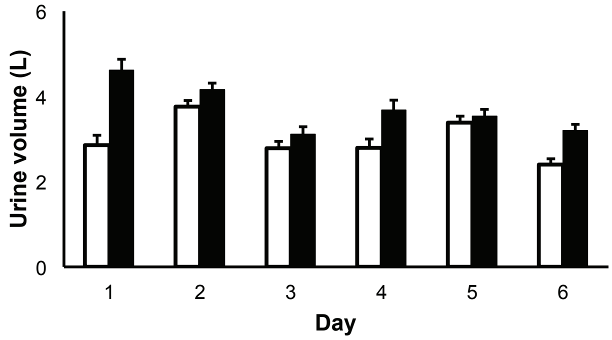
## Results

One sheep in the Plantain group was removed from the trial due to failure to eat the allocated feed. On the first day that sheep were introduced to the study (Day 1) all animals had very high urine osmolality (see Table 2), unique to that day, and the urine volumes of Plantain sheep exceeded those of Ryegrass sheep on average by more than 1.5 L (Table 2). This was significantly different ($P < 0.05$) from all subsequent measurements, therefore, results from the Day 1 day have been analysed as a separate data set from those of the other five days (Days 2-6). Some measurements of feed and faeces from Day 7 of the study were inadvertently lost, so the data analysis ends at Day 6. Daily intake of water was about 4 L for all sheep and generally comparable between both groups of sheep (Table 2). Sheep in both groups lost weight during the experiment with Plantain sheep losing more ($P < 0.001$) than Ryegrass

**Table 1** Haematological data (mean ± SEM) for sheep fed ryegrass (Ryegrass) or plantain (Plantain) recorded from blood collected on Day 7 of the study. Number of animals per group in parentheses. Probability of between-diet difference (as determined by Student's *t*-test) is indicated.

| | Ryegrass (n = 8) | Plantain (n = 7) | Significance P value |
|---|---|---|---|
| Red blood cells ($x10^7/\mu L$) | 1.69 ± 0.09 | 1.63 ± 0.1 | 0.668 |
| Packed cell volume (%) | 35 ± 1.0 | 37 ± 1.1 | 0.140 |
| Plasma sodium (mmol/L) | 147 ± 0.4 | 147 ± 0.4 | 0.420 |

**Figure 1** Mean daily urine volume of sheep fed ryegrass (n = 8, open bars) or plantain (n = 7, solid bars) commencing at the start of Day 1. Data are shown for the first six days. Vertical lines show + SEM.




sheep (mean ± SEM loss for 7 days, 3.7 ± 0.2 and 1.0 ± 0.6 kg, respectively). However, based on haematological data (Table 1) from blood obtained at the end of the study (red blood cell count, packed cell volume, plasma sodium concentration) there was no evidence of dehydration.

Plantain sheep lost more water in urine each day than the Ryegrass sheep (Table 2, Figure 1). Initially Plantain sheep excreted urine with similar specific gravity and osmolality to that from Ryegrass sheep (Table 2), so the total osmols excreted per day exceeded that of Ryegrass sheep because of the greater volume of urine excreted. However, on the ensuing five days, Plantain sheep excreted urine that was slightly more dilute (lower specific gravity and osmolality) but the total daily osmotic content was not different from that of Ryegrass sheep urine (Table 2). Ryegrass sheep consumed more dry matter (about 200 g per animal per day) than Plantain sheep (Table 2) and excreted more faecal water (about 150 ml per sheep per day in the final 5 days, Table 2).

The Plantain sheep had a very much lower (P < 0.001) daily water balance than the Ryegrass sheep (mean ± SEM daily average for Days 1 - 6, 27 ± 50 and 533 ± 81 mL, respectively).

**Table 2** Measurements of inputs and outputs (mean ± SEM) for sheep fed ryegrass (Ryegrass) or plantain (Plantain) for seven days. Number of animals per group is given in parentheses. Probability of between-diet difference (as determined by Student's *t*-test) is indicated.

| | Ryegrass (n = 8) | Plantain (n = 7) | Significance P value |
|---|---|---|---|
| Day 1 | | | |
| Water in feed (L) | 4.0 ± 0.06 | 3.7 ± 0.07 | 0.042 |
| Dry matter in feed (g) | 839 ± 12 | 634 ± 13 | <0.001 |
| Urine volume (L) | 2.9 ± 0.2 | 4.6 ± 0.2 | <0.001 |
| Fresh faecal weight (g) | 612 ± 59 | 545 ± 63 | 0.458 |
| Faecal water (mL) | 450 ± 52 | 399 ± 56 | 0.519 |
| Urine specific gravity | 1.035 ± 0.001 | 1.037 ± 0.002 | 0.458 |
| Urine osmolality (mmol/kg) | 1147 ± 44 | 1106 ± 47 | 0.544 |
| Total osmols excreted (mmol) | 3290 ± 379 | 5171 ± 405 | 0.005 |
| Days 2-6 (Means are the average daily value) | | | |
| Water in feed (L) | 3.9 ± 0.05 | 4.0 ± 0.05 | 0.122 |
| Dry matter in feed (g) | 686 ± 9.1 | 463 ± 8.6 | <0.001 |
| Urine volume (L) | 3.0 ± 0.08 | 3.5 ± 0.09 | 0.002 |
| Fresh faecal weight (g) | 494 ± 21 | 284 ± 21 | <0.001 |
| Faecal water (mL) | 360 ± 21 | 198 ± 20 | <0.001 |
| Urine specific gravity | 1.014 ± 0.001 | 1.009 ± 0.001 | 0.010 |
| Urine osmolality (mmol/kg) | 499 ± 36 | 326 ± 37 | 0.011 |
| Total osmols excreted (mmol) | 1491 ± 134 | 1093 ± 131 | 0.064 |

## Discussion

These results show that the ingestion of leaves from the narrow-leaved plantain (*Plantago lanceolata* L.) has increased urine volume of sheep compared with those consuming ryegrass and it was achieved without access to drinking water. In this instance, the loss of additional water in urine by sheep eating plantain, presumably at the expense of water reserves within their body, is strong evidence for the presence of a diuretic property contained within the plantain diet. This is the first scientific study to report a diuretic effect following ingestion of this plantain by sheep. A prior study (Wilman & Derrick 1994) showed increased urine production of sheep eating plantain compared with those on a diet of ryegrass but the lack of information on intakes makes it difficult to reach any conclusion about whether a diuresis occurred. The other study (Deaker et al. 1994) simply recorded an increase in kidney weight of sheep consuming plantain. Although there is evidence from trials using laboratory rats of a diuretic property of plantain (Cáceres et al. 1987), this was for a different plant species, the broadleaf or greater plantain (*Plantago major*). The present finding is certainly the first to determine that the narrow-leaved plantain has diuretic activity when ingested by sheep and, because this herb is being established as an alternative species in New Zealand pastures, may have implications for the impact of urine from livestock on the environment.

On the first day of the trial, Plantain sheep had a much greater urine volume (about 1.5 L) than the Ryegrass sheep. This could be the result of the dramatic diuretic effect resulting from consumption of plantain and the plentiful water reserves of the sheep, e.g., rumen fluid, at the outset. Thereafter, the diuretic effect, although still considerable (i.e., about 0.5 L per day), was probably offset by a decline in water reserves plus homeostatic responses engendered by the alteration in kidney function. Further studies, in which the size of pools such as total body water, extracellular fluid volume and rumen volume are measured, will be required to confirm this suggestion. The high urine osmolalities (*circa* 1100 mmol/kg) recorded on the first day, in comparison with the lower values thereafter (*circa* 300 to 500 mmol/kg), must have been a carryover from the outdoor grazing situation of these sheep prior to the study and were not affected by the first day's feeding. During the remainder of the study, Days 2 to 6, the Plantain sheep have generally produced a greater volume of urine that was slightly diluted (lower specific gravity and osmolality) but held no more total osmols per day than that from the Ryegrass sheep. This indicates that the Plantain sheep excreted more water *per se* than the Ryegrass sheep during these 5 days, as borne out by their much lower water balance. Although there was no evidence of dehydration (haematology and plasma sodium data) in the Plantain sheep, it is likely that these animals would have drunk extra water if it had been available, and their urinary volumes would have been even greater than those recorded here.

The experimental approach used in this study, to match water intakes of the two groups of sheep, was largely achieved. However, there was a slight imbalance in terms of dry matter, with Ryegrass sheep ingesting more in their feed and producing greater weight of faeces and faeces water contents than Plantain sheep. Nevertheless, the extra feed consumed as dry matter by the Ryegrass sheep has not altered urine volumes or faeces water losses sufficiently to account for the much larger urinary water loss of Plantain sheep. Also, the slightly higher evaporative losses required for metabolism of the extra dry matter are likely to have been offset by the water contribution from its metabolism. Thus, any effect of the imbalance in dry matter content of the two diets would likely mitigate rather than contribute to the main finding of this study – that the sheep consuming plantain produced a greater volume of urine.

In physiological terms, these results indicate that consumption of plantain has produced a 'water diuresis'. This is where there is a reduction in reabsorption of water from the fluid present in the distal convoluted tubules and collecting ducts of the kidneys, leading to a greater urine volume that is not matched by an increased osmotic load – as observed in the present study. Two possibilities exist to explain this – either, (1) interference with release of vasopressin, or (2) blockade of action of vasopressin via its receptors. Such effects may be attributable to bioactive components actually present in plantain leaves or result from compounds derived from ruminal or post-ruminal metabolism of a precursor contained in the plant.

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## References

Cáceres A, Girón LM, Martínez AM. 1987. Diuretic activity of plants used for the treatment of urinary ailments in Guatemala. Journal of Ethnopharmacology 19: 233-245.

Deaker JM, Young MJ, Fraser TJ, Rowarth JS. 1994. Carcass, liver and kidney characteristics of lambs grazing plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*). Proceedings of the New Zealand Society of Animal Production 54: 197-200.

Rumball W, Keogh RG, Lane GE, Miller JE, Claydon RB. 1997. 'Grasslands Lancelot' plantain (*Plantago lanceolata* L.). New Zealand Journal of Agricultural Research 40: 373-377.

Wilman D, Derrick RW. 1994. Concentration and availability to sheep of N, P, K, Ca, Mg and Na in chickweed, dandelion, dock, ribwort, and spurrey, compared with perennial ryegrass. Journal of Agricultural Science, Cambridge 122: 217-223.

Tamura Y, Nishibe S. 2002. Changes in the concentrations of bioactive compounds in plantain leaves. Journal of Agricultural and Food Chemistry 50: 2514-2518.